

Listing of Claims

What is claimed is:

1-21. (Canceled)

22. (Currently amended) ~~The process of Claim 21~~ A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising expressing the nucleic acid of a host cell comprising:

a nucleic acid sequence comprising:

Px-Sx-Bn-(ZR)-Hir(AsmR)-protein(Y)-T

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

Bn is 1-15 codons, when n is an integer from 1 to 15, or a chemical bond, when n=0;

Z is a codon for lysine or arginine;

R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

Asm is a chemical bond, when m=0, or 1-10 codons, when m is an integer from 1 to 10;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; and

T is an untranslated expression-enhancing nucleic acid sequence,

as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally to form the fusion protein in a fermentation supernatant of a cell culture and isolating said fusion protein from said fermentation supernatant,

wherein said isolating the fusion protein comprises adjusting the pH of said fermentation supernatant to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

23. (Currently amended) ~~The process of claim 14 further~~ A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising:

expressing the nucleic acid of a host cell comprising:

a nucleic acid sequence comprising:

Px-Sx-Bn-(ZR)-Hir(AsmR)-protein(Y)-T

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

Bn is 1-15 codons, when n is an integer from 1 to 15, or a chemical bond, when n=0;

Z is a codon for lysine or arginine;

R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

Asm is a chemical bond, when m=0, or 1-10 codons, when m is an integer from 1 to 10;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; and

T is an untranslated expression-enhancing nucleic acid sequence,

as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally to form the fusion protein in a fermentation supernatant of a cell culture and

(A) separating the fermentation supernatant from the host cell;

(B) culturing the host cell in fresh medium;

(C) separating the resulting supernatant from the host cell;

(D) repeating (B) and (C) several times; and

(E) isolating the fusion protein from the aforementioned supernatants by adjusting the pH of said supernatants to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

24. (Currently amended) ~~The process of claim 21~~ A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising expressing the nucleic acid of a host cell comprising:

a nucleic acid sequence comprising:

Px-Sx-Bn-(ZR)-Hir(AsmR)-protein(Y)-T

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

Bn is 1-15 codons, when n is an integer from 1 to 15, or a chemical bond, when n=0;

Z is a codon for lysine or arginine;

R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

Asm is a chemical bond, when m=0, or 1-10 codons, when m is an integer from 1 to 10;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; and
T is an untranslated expression-enhancing nucleic acid sequence,

as part of the host cell chromosome, as part of a mini-chromosome, or extra-
chromosomally to form the fusion protein in a fermentation supernatant of a cell culture and
isolating said fusion protein from said fermentation supernatant,

wherein the isolating the fusion protein comprises precipitating the fusion protein from the fermentation supernatant, and further comprising releasing the protein encoded by protein(Y) from the fusion protein and concentrating said protein encoded by protein(Y) by microfiltration, hydrophobic interaction chromatography, ion exchange chromatography, or a combination of such procedures.

25-29. (Canceled)

30. (Currently amended) ~~The process of claim 21~~ A process of fermentative
production of a hirudin-mini-proinsulin fusion protein comprising expressing the nucleic acid
of a host cell comprising:

a nucleic acid sequence comprising:

Px-Sx-Bn-(ZR)-Hir(AsmR)-protein(Y)-T

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

Bn is 1-15 codons, when n is an integer from 1 to 15, or a chemical bond, when n=0;

Z is a codon for lysine or arginine;

R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

Asm is a chemical bond, when m=0, or 1-10 codons, when m is an integer from 1 to 10;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; and

T is an untranslated expression-enhancing nucleic acid sequence,

as part of the host cell chromosome, as part of a mini-chromosome, or extra-
chromosomally to form the fusion protein in a fermentation supernatant of a cell culture;

isolating said fusion protein from said fermentation supernatant, and

~~further comprising~~ releasing mini-proinsulin by treating said fusion protein with trypsin and carboxypeptidase B.

31. (Currently amended) ~~The A~~ nucleic acid sequence ~~of claim 1 in which~~ comprising:

Px-Sx-Bn-(ZR)-Hir(AsmR)-protein(Y)-T

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

Bn is 1-15 codons, when n is an integer from 1 to 15, or a chemical bond, when n=0;

Z is a codon for lysine or arginine;

R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

(AsmR), taken together, is an arginine codon

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; and

T is an untranslated expression-enhancing nucleic acid sequence.

32. (Currently amended) The nucleic acid sequence of claim + 31 in which (AsmR), taken together, encodes SEQ ID NO: 12 (Gly-Asn-Ser-Ala-Arg).

33. (Currently amended) The nucleic acid sequence of claim + 31 in which Px is a yeast ADH2 promoter, Sx is an a factor leader sequence, and Hir encodes hirudin or lepirudin, (AsmR), taken together, is either an arginine codon or encodes SEQ ID NO:12 (Gly-Asn-Ser-Ala-Arg).

34. (Canceled)

35. (Currently amended) The nucleic acid of Claim ~~34~~ 31 wherein Hir is a nucleic acid sequence encoding lepirudin which has been prepared recombinantly.